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REVIEW

A Review of Meibography

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ABSTRACT

Meibomian gland dysfunction is one of the most common causes of dry eye resulting in morphology changes to the meibomian glands. Meibography provides an in vivo means to assess the structure of the meibomian gland. Over the past 40 years, meibography has undergone significant development regarding its application to research and clinical practice. This review describes the evolution of the various meibography techniques, grading methods, and their diagnostic relevance.
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Key Words: meibography, meibomian glands, meibomian gland dysfunction, dry eye, imaging

Meibomian gland dysfunction (MGD) is one of the most common abnormalities in ophthalmic practice[1] resulting in an abnormality of the tear film lipid layer[2] and evaporative dry eye.[1–5] MGD is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion.[6] This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease.[6]

MGD results in stasis of meibum inside the glands, dilation of the ductal system, and loss of glandular tissue (gland dropout).[2] Meibography provides an in vivo means to assess the structure of the meibomian gland. When using this method, the structure of the meibomian glands, including the ducts and acini, can be observed (Fig. 1).[7–15]

Meibography provides photographic documentation of the meibomian gland under specialized illumination techniques.[16] Normal meibomian glands appear as grapelike clusters with acini that are hypoilluminescent.[15] Ducts and orifices transmit light and appear as hyperilluminescent regions surrounded by the gland acini. There are two different types of meibography: transillumination of the everted lid (Fig. 2)[10,17,18] and direct illumination (Fig. 1B), named non-contact meibography.[14,19–21]

Transilluminating Meibography

In the transillumination technique, the eyelid is everted over a light source (Fig. 2).[10,12,22] The most basic version uses white light, for example, from a Finoff transilluminator. This is applied to the cutaneous side of the everted eyelid and allows observation from the palpebral conjunctival surface. We believe that this technique was originally reported by Tapie[23] in 1977 with later studies reporting its viability for visualizing the structure of the meibomian gland. Tapie used a diaphanoscope with a red light filter to transilluminate the lids and a slitlamp microscope to observe the meibomian glands. He used red light to overexpose the vessels of the tarsal conjunctiva. Interestingly, Tapie[23] also described the evaluation of the conjunctival tarsi by ultraviolet light (Fig. 3). He mentioned "Sometimes it's helpful to alternate the light of the diaphanoscope with ultraviolet Wood's light when observing the tarsal conjunctiva, since the glandular lobules show a slight fluorescence in green coloration . . . The ultraviolet light often highlights the content of the dilated central channels: it's outlined against the rest of the tarsal conjunctiva by its greater fluorescence, and this proves beyond doubt that the secretion of these glands is hereby altered. . . ."[23] To our knowledge, this is the only article reporting this effect.

Tapie also captured images of glands when taken by infrared (IR) photography using an IR Kodak film in combination with his standard diaphanoscope. Since he discussed the standard parameters of the diaphanoscope, we assume that he used white light as his light source in combination with IR photography. If IR photography was preceded with a slitlamp microscope, it was not explicitly mentioned.

Baum[24] commented on Tapie's method. "I have attempted, but have been unable, to distinguish these differences in most patients. Since the technique is simple to execute and the rapid diagnosis of Meibomian gland disease of significant value, I call this article to the readers' attention and trust that others will validate Dr. Tapie's interesting findings."[24]

Later, Jester et al.[15] adapted the biomicroscopic and photographic techniques to improve upon Tapie's technique when

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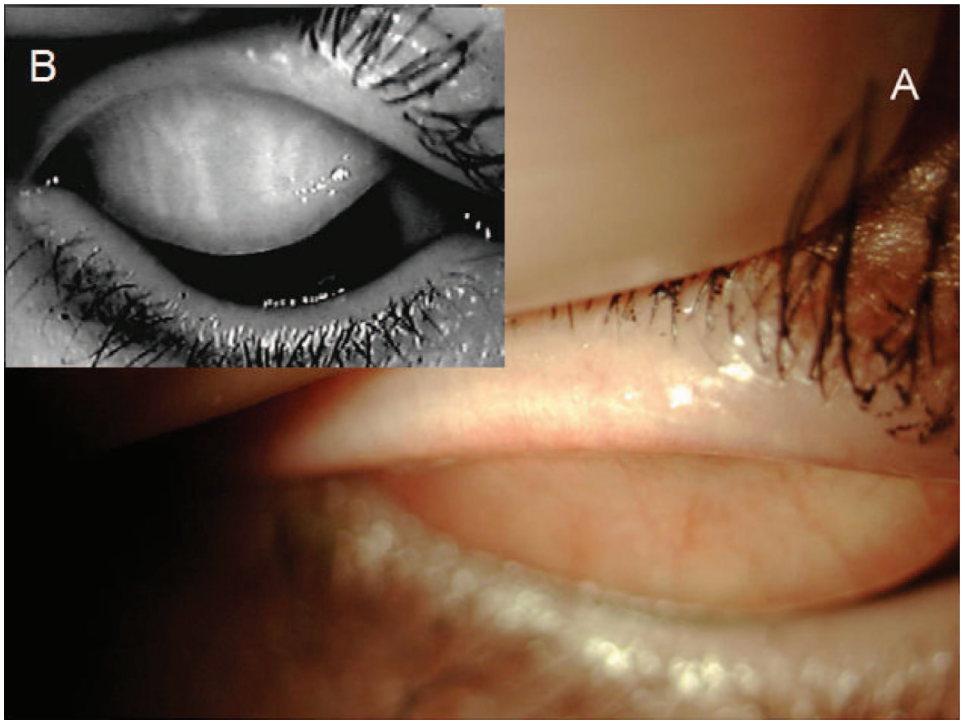

FIGURE 1.

(A) Same everted eyelid observed by slitlamp microscope and (B) non-contact meibography. A color version of this figure is available online at www.optvissci.com.

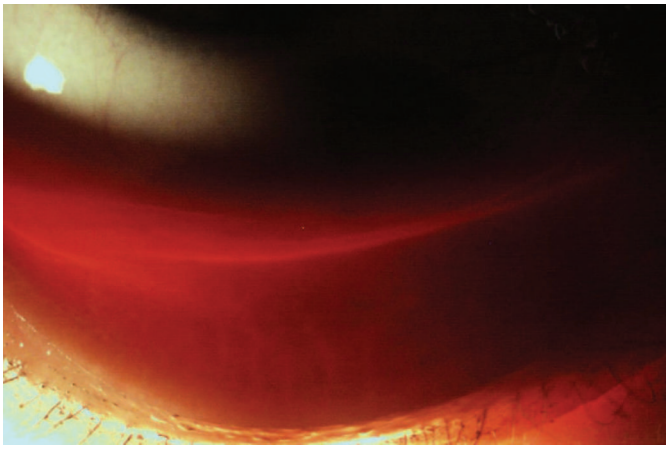

FIGURE 2.

Transillumination of the everted eyelid using a white light source. A color version of this figure is available online at www.optvissci.com.

observing rabbits.[15] They evaluated the lids of rabbits by gross examination with an OS 3001 transilluminator (white light; Medical Instruments Research Associates, Inc., Boston, MA) and a Nikon 20-diopter indirect lens. They examined two groups including one group that received one drop of topical 0.5% epinephrine hydrochloride in each eye and second group that received 2% epinephrine (in addition to control animals that did not receive drugs). The rabbits were continued on this regime over a 6-month follow-up period. Morphologic changes in the meibomian glands were documented by using transillumination (white light) with a Zeiss photo-slitlamp microscope with high-speed IR KODAK film (HIE 135-20).[15] Robin et al.[25] also showed the usefulness of this technique in evaluating MGD in humans. Subsequently, many other groups have used the transillumination IR techniques[10,17,18] in meibomian gland observation.

In 1991, Mathers et al.[13] observed patients with symptoms of chronic blepharitis using meibomian gland expression, tear osmolarity, Schirmer's test, and meibography. Meibography was obtained by the technique of Jester et al.[15] They used a fiber-optic light probe transilluminator (Medical Research Instruments, Inc., Cambridge, MA) and IR photography (Kodak high-speed black and white IR film). Even though this article stated in the methods section "all patients were photographed with infrared transillumination of the meibomian gland as previously described,"[15] it does not appear that the use of an IR light source was actually employed as they explicitly cited the work of Jester et al.[15] and Jester was a co-author of this paper. However, this appears to be the first time that this general technique was named "meibography."

Analog IR photography is expensive and the result of the observations is only known after the film is developed. This also prohibits the observer from controlling the quality of the image taken. Mathers et al.[17] described implementing video technology in 1994 to overcome the expensive use of IR film and to provide better efficiency than still IR film. They used a one-chip IR video camera, a hand-held transilluminating light source, and a video monitor.[17] A super VHS recorder was utilized to maintain a visual record of each individual's meibomian glands.[17] They compared IR photographs of meibomian glands taken with a standard ophthalmic camera and slitlamp microscope with the videocassette recording of meibography. Despite the potential loss of resolution due to conversion from tape to film, visualization of the glands was higher quality in the video image compared with the IR photographs.[17]

Nichols et al.[18] used digital video meibography imaging in the assessment of meibomian glands, including the assessment of the

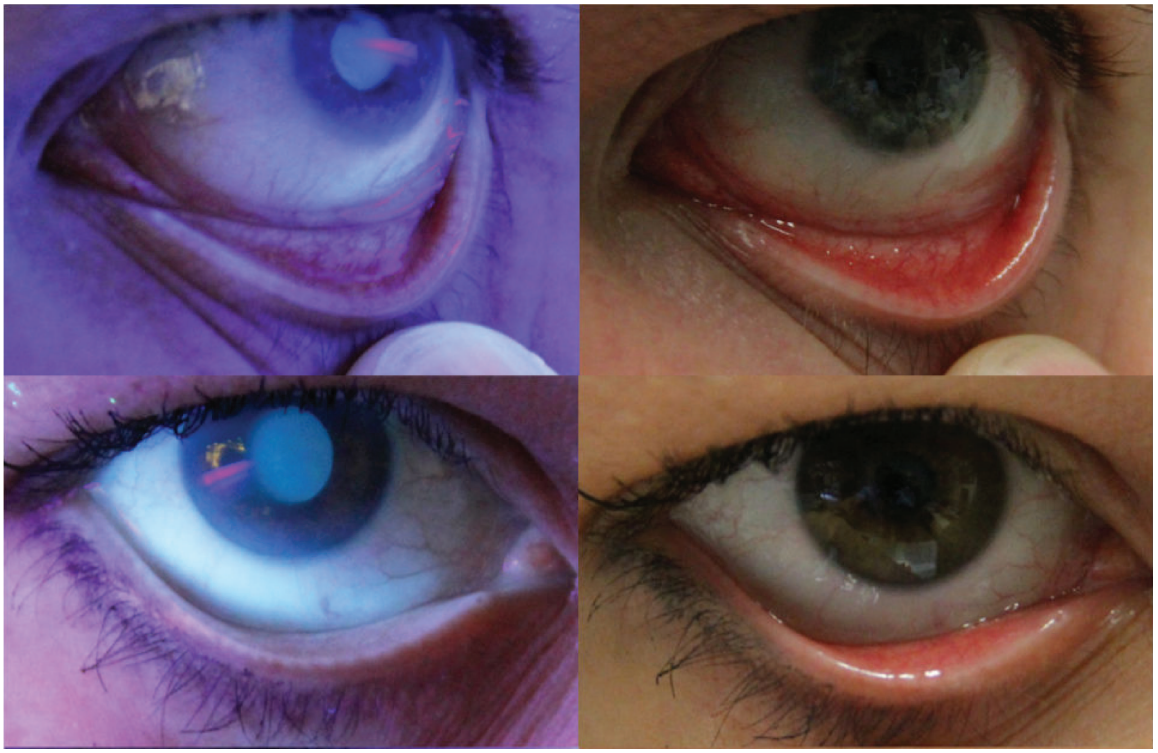

FIGURE 3.

Observation of the meibomian glands using ultraviolet Wood's light as described by Tapie.[23] A color version of this figure is available online at www.optvissci.com.

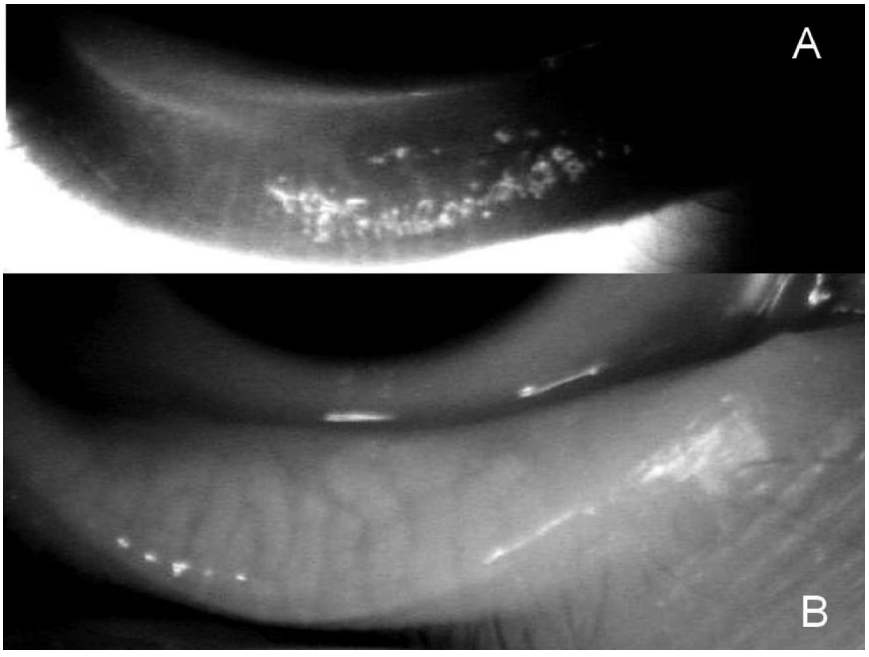

FIGURE 4.

Contact meibography (A) using a white light transilluminator and the Portable Non-Contact Meibography (PNCM; IR lighting switched off) vs. non-contact meibograpy (B) by the PNCM.

repeatability of associated grading scales for these images. The lower eyelid was transilluminated using a Dolan-Jenner transilluminator and fiber-optic light guide using near-IR light (wavelength 650–700 nm). IR light is usually divided into three spectral regions: near-, mid-, and far-IR. The boundaries between the near-, mid-, and far-IR regions are not agreed upon and can vary. However, near-IR light is mostly described as starting at a wavelength of 700 nm.[26] Images were recorded using a Hitachi KP-M2R near-IR 1-chip CCD camera from the central eyelid at 10× slitlamp microscope magnification. This enabled the capture

of up to 15 individual glands in the central image sequences.[18] To our knowledge, this was the first time the use of an IR light source was explicitly mentioned. The spectrum of the white transilluminator used in the other studies is not exactly known, but it is well known that white light sources contain some IR illumination making IR photography possible. The advantage of white light is that it is visible to the human eye, while IR light is not, making the white light source needed when using photography via film. However, white light is not needed when using digital IR video recording given the real-time viewing via a computer and monitor. We believe that illumination with adequate near-IR light probably results in better digital IR images than standard white light by reducing the CCD pixel exposures to non-relevant light sources.

Yokoi et al.[10] also described the use of IR light (wavelength, 850 nm). They developed a new IR probe for meibography to overcome the difficulties associated with the traditional meibography technique, and in particular, the light source used for transillumination.[10] The manipulation of the eyelid required to carry out meibography with the traditional probe can be difficult and involves eversion of the eyelid by the narrow tip of the probe; additional disadvantages are the brightness and heat produced by the probe's light, both of which might be associated with discomfort for patients during the examination.[10] In addition, the area transilluminated by the traditional probe is small, making visualization of the entire meibomian structure within the eyelid difficult.[10]

Non-Contact Meibography

In the non-contact technique, a camera and IR light source do not touch the patient during the meibography procedure.[14] Non-contact meibography was first introduced by Arita et al. in 2008.[14,19–21] Their non-contact meibograph[14] consists of a slit-lamp microscope equipped with an IR charge-coupled device video camera and an IR transmitting filter[14] to allow the observation of the everted lid (but without contact with a light probe with the everted lids being directly illuminated by IR light). The light and dark contrast of the meibomian glands is opposite that of the transillumination technique (Fig. 4) in that they appear light instead of dark.

The potential advantage of this technique is that it may be more comfortable for patients, in addition to the fact that this system is now commercially available in some markets as additional equipment for the Topcon slitlamp microscope (Topcon Cooperation, Tokyo, Japan). A normal IR-CCD video camera including an IR

FIGURE 5.

Digital photography (left) with use of an IR filter (Canon G10, f = 4.0, t = 8 s, and ISO = 400) compared with the same scenery (right) without IR filter (f = 4.0, t = 1/500, and ISO = 400).

light source was reported in 2011[8] to be a useful alternative for non-contact meibography. This set-up consisted of an IR CCD video-camera (pixel: PAL: 628(H) × 582(V), ¼″ CCD Sensor, 802CHA CCD; Shenzhen LYD Technology Co. Ltd, Shenzhan, China) and near focus adaptation by a +20 diopter lens and was named the Portable Non-Contact Meibography (PNCM). Such cameras are readily used as security or backup cameras. Many common ophthalmic instruments like topographers also have built-in IR cameras combined with IR light sources to be designed for pupillometry. It has been also demonstrated that these devices can be used in meibography.[27–29] However, minor optical and software modifications are required.[27,28] Commercially available instruments are now available in some markets through CSO (Costruzione Strumenti Oftalmici, Florence, Italy) and bon Optic VertriebsgmbH (Lübeck, Germany),[30] followed by Oculus Optikgeräte GmbH (Wetzlar Germany).[29,31] This might make non-contact IR meibography more accessible and more used in clinical practice.

IR Photography

IR photography is the imaging of IR light. Analog cameras require IR sensitive film (spectral-sensitivity ~250–950 nm), but in digital cameras, the silicone-based CCD-chip is inherently IR sensitive. Silicon's maximum sensitivity to light is at about 850 nm. Therefore, they are usually covered by a green barrier filter to prevent otherwise marked chromatic effects that would degrade image quality.

To filter out the unused portion of the white light spectrum, IR filters are recommended in IR photography in general. These filters enhance the IR effect of the IR film, since these filters absorb the ultraviolet radiation and blue light to which the IR film is also sensitive. However, in the aforementioned meibography techniques, eyelids were transilluminated with a white light source (e.g., incandescent bulbs), which often peaks in spectral sensitivity ~500 nm, and the use of an IR filter was not mentioned.[13,15,23,25] Furthermore, the eyelid acts as a red filter itself absorbing these wavelengths of light. As all images were taken by black/white IR, the omission of an additional IR filter was obvious, especially because long film exposure times would have been required.

In digital photography, an IR filter is vital, although in many cameras, some IR light still transmits through the filter; therefore, they can also be used for IR photography (Fig. 5). Other systems can be switched into night modes in which the green IR barrier filter is automatically removed. Pure digital IR cameras do not have such filters and are consequently highly sensitive to near-IR light due to the silicone CCD chip's properties, which use the low light and mostly illuminate objects by built-in IR light emitting diodes. IR photography gives further information of material properties (e.g., in nature; for example, damage to a forest).[32–35] The spectrum of light visible to humans might partly be absorbed by leaves, while IR light is reflected, as chlorophyll is IR hyperreflective (Fig. 5).[35] This effect can be transferred to the evaluation of meibomian glands.

It is possible that application of different light spectra might give additional information also in meibography. IR techniques enhance visibility of gland morphology, as glands appear to be IR hyperreflective/hypoilluminescent. Knowing what component of the meibomian gland is IR hyperreflective (e.g., tissue, cell type, and secretion) might be insightful as to the assessment of the glands via meibography. To our knowledge, it is not known whether intensity of IR reflection is correlated to gland secretion, alteration of secretion, or gland tissue, but we suspect that there are no changes given the relatively little energy associated with long-wavelength light such as IR. Jester et al.[15] reported correlation between 2% epinephrine-induced cystic change of meibomian glands (rabbit) and drop out of glands observed by IR photography. He also commented on the correlation between dark spots on the photographs and hyperkeratinization of the glands' orifices. Tapie[23] reported the importance of altering the light spectrum in gland observation (red light vs. ultraviolet light). Therefore, further investigation of effects of the light spectrum in the observation of gland morphology and secretion might be worthwhile.

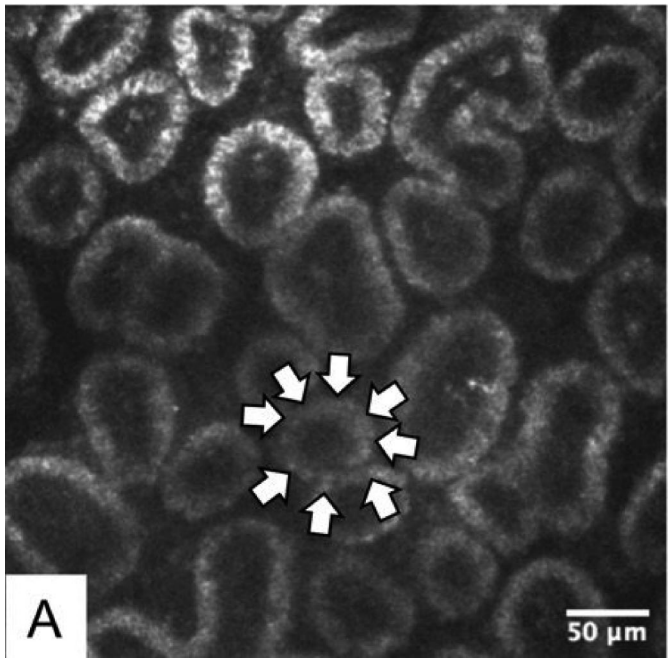

FIGURE 6.
In vivo confocal microscopy of the eyelid from a representative normal subject. White arrows depict a typical acinar unit. Note the presence of numerous and compact acinar units. Reproduced with permission from Ibrahim OM, Matsumoto Y, Dogru M, Adan ES, Wakamatsu TH, Goto T, Negishi K, Tsubota K. The efficacy, sensitivity, and specificity of in vivo laser confocal microscopy in the diagnosis of meibomian gland dysfunction. Ophthalmology 2010;117:665–72.

Other Techniques to Image the Meibomian Glands

In vivo confocal laser microscopy is a technology that is useful as a supplementary diagnostic tool for in vivo assessment of the histopathology of many ocular surface disorders.[36,37] Although confocal microscopy was not designed with meibographic imaging in mind, it should still be mentioned. However, it is an invasive technology, since a drop of oxybuprocaine chlorohydrate 0.4% and an ophthalmic polyacrylic gel 0.2% need to be instilled into the conjunctival fornix before each examination and the center of the objective lens of the confocal laser microscopy—covered with a sterile cap—is applanated onto the center of the eyelid margin.[37] To our knowledge, Messmer et al.[36] were the first to describe in

vivo confocal microscopy in the observation of meibomian glands. In vivo confocal microscopy can effectively demonstrate the morphological changes of the meibomian glands in patients and give additional information to meibography (e.g., glandular acinar density and acinar unit diameter; Fig. 6).[9,38,39] Finally, although not reported in the literature, functional magnetic resonance imaging can also be used to image the meibomian glands (one of the authors has observed this), although there are many challenges associated with this technique that likely do not warrant its further use.

Grading of Meibomian Glands

There is no gold standard in the classification of meibomian glands. For example, some have analyzed number of glands,[18,22] other percentage of partial glands,[18] gland dropout,[8,13,14] duct dilation,[13,25] or hypertranslucent cysts and scars (chalazia).[13] However, only a few detailed grading scales are reported (Table 1).

Pflugfelder et al.[40] graded gland dropout by a four-grade scale. Another similar grading scale describes the meibography images using a gestalt scale.[18] Meibomian glands are classified if they are complete or partial.[18] Complete meibomian glands are those that traverse the lid linearly about 3 to 4 mm, although there can be tortuosity associated with full glands.[18] Tortuosity is more distinct in the upper eyelid meibomian glands than the lower eyelid glands.[41] Glands that do not traverse the lid fully or are found in irregular clumps are named "partial" meibomian glands. Another grading scale of the same group evaluate the number of complete meibomian glands in the image.[18] Arita et al.[14] scored changes in meibomian glands using a four-grade scale describing area of meibomian gland loss. McCann et al.[22] defined meibomian gland dropout by the total number of glands absent. If one-half was missing, this was counted as 0.5 gland dropout, and when using this grading scale, significant differences in tear film physiology and meibomian gland (MG) function in patients with blepharitis were analyzed.[22] Computerized classification of ocular signs is increasingly applied in research, including meibography as well.[8,41–48] Computerized grading of meibomian gland morphology was reported, measuring "area of loss of MG," "thickness of MG," and "bent of MG" (Fig. 7).[8,41] Criteria were measured applying ImageJ 1.42q (Wayne Rasband, National Institute of Health, Bethesda, MD).

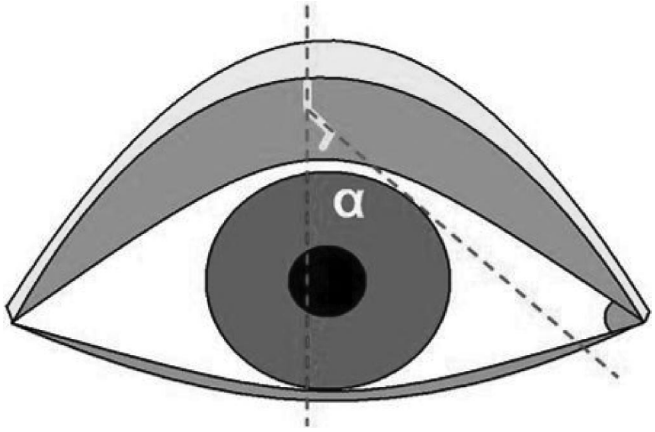

FIGURE 7.
Schematic showing an illustration of the determination of the bent angle (α) that can be associated with the meibomian glands.[41] A color version of this figure is available online at www.optvissci.com.

To our knowledge, repeatability of examiners and grading scales was analyzed only by Nichols et al.[18] (gestalt scale and partial glands scale) showing modest repeatability. Nichols et al.[18] concluded the gestalt scale to be slightly superior to the gland counting approach. However, one can assume that computerized grading improves sensitivity[41] and repeatability. Pult and Riede-Pult[49] analyzed repeatability of computerized grading in comparison to a four-grade meiboscale and a five-grade meiboscale (Fig. 8). Interobserver and intraobserver agreement was best in computerized grading (Fig. 9), followed by the five-grade meiboscale and the least repeatable being the four-grade meiboscale.

Diagnostic Relevance

Mathers et al.[13] demonstrated that an objective analysis of meibomian gland function can be used to assess chronic blepharitis and define subsets of blepharitis (which includes posterior bleph-

TABLE 1.
Different grading scale in meibography

Pflugfelder et al.[40]	**Grade 0** No gland dropout	**Grade 1** 33% gland dropout	**Grade 2** 34%–66% gland dropout	**Grade 3** More than 66% gland dropout	
Nichols et al.[18]	**Grade 1** No partial glands	**Grade 2** Less than 25% of the image contains partial MG	**Grade 3** Between 25% and 75% of the image contains partial MG	**Grade 4** More than 75% of the image contains partial MG	
Arita et al.[14]	**Grade 0** No loss of meibomian glands	**Grade 1** Area loss less than one third of the total MG area	**Grade 2** Area loss between one-third and two-thirds of the total MG area	**Grade 3** Area loss more than two-thirds of the total MG area	
Pult and Riede-Pult (submitted)	**Grade 0** Area of loss 0%	**Grade 1** Area of loss <25%	**Grade 2** Area of loss 25%–50%	**Grade 3** Area of loss 51%–75%	**Grade 4** Area of loss >75%

MG, meibomian glands.

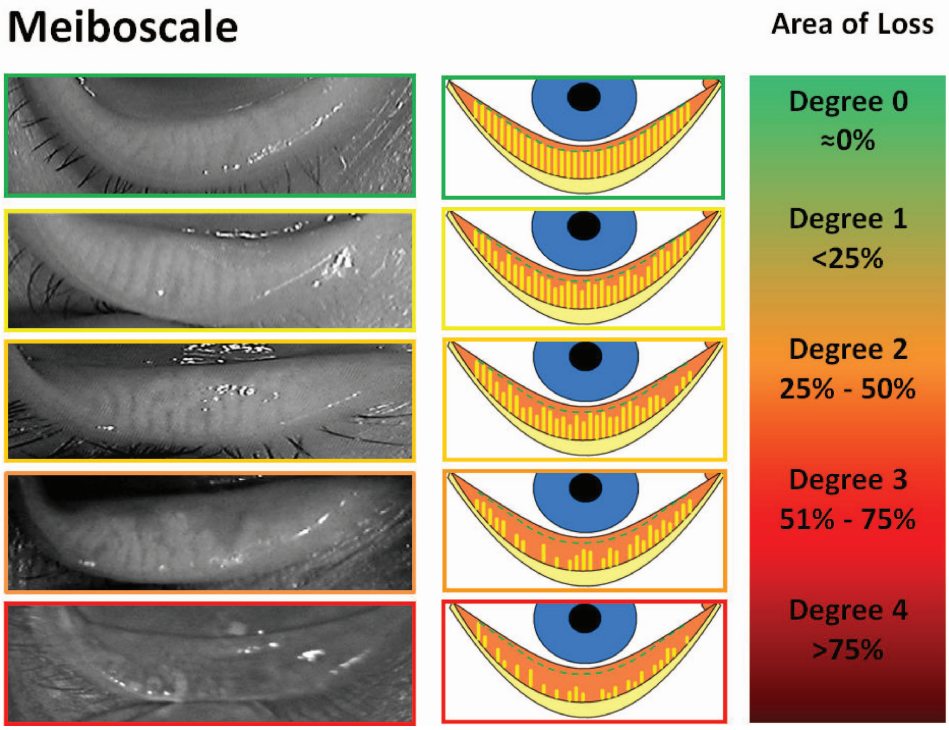

FIGURE 8.

Five-grade pictorial, photographic, and verbal Meiboscale (Pult and Riede-Pult, submitted). A color version of this figure is available online at www.optvissci.com.

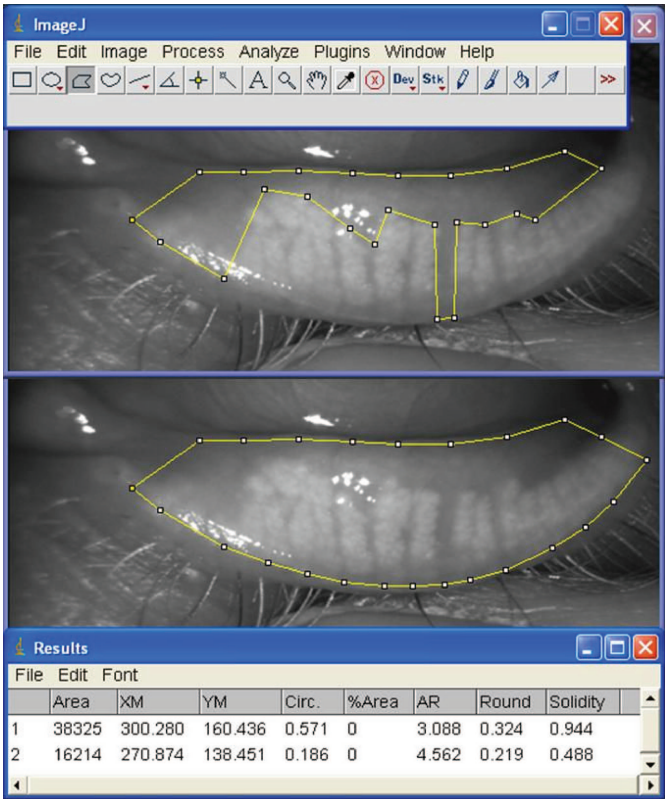

FIGURE 9.

Computerized analyses of the area of gland loss.[8] A color version of this figure is available online at www.optvissci.com.

aritis and MGD) with measurable differences. They also supported the significance of MGD on tear osmolarity and the evaporative state of the eye. McCann et al.[22] evaluated blepharitis by tear physiology, evaporimetry, interferometry, meibomian gland expression, and meibography. All the tests employed were suggested to be useful as single tests in the diagnosis of MGD (blepharitis), with meibomian gland dropout of the entire lower eyelid offering the greatest effectiveness as a single measure.[22] To evaluate diagnostic criteria for obstructive MGD, Arita et al.[20] used three parameters (symptom score, lid margin abnormality score, and meibomian gland morphologic change scores) for differentiating obstructive MGD from aqueous deficiency dry eye (ADDE). Although the criteria were moderately reliable for differentiating patients with obstructive MGD from those with ADDE, they do not provide comprehensive diagnostic tools for differentiating MGD, ADDE, and healthy individuals.[20] They recommended the need of other parameters such as the Schirmer test value and the meibum score to enhance their reliability for differentiating MGD and ADDE.

Ibrahim et al.[9] evaluated the usefulness of in vivo confocal microscopy parameters [meibomian gland (MG), acinar longest diameter (MGALD), MG acinar shortest diameter (MGASD), inflammatory cell density (ICD), and MG acinar unit density (MGAUD)] in the diagnosis of MGD. They suggested confocal microscopy to have potential to diagnose MGD with high sensitivity and specificity. The in vivo confocal microscopy-based diagnostic parameters correlated significantly with tear film stability, tear evaporation rate, and corneal and conjunctival staining.[9]

Significant correlations between area of loss of MG (MGL) and lipid layer, non-invasive break-up time, and dry eye symptoms were reported by Pult and Riede-Pult.[8] MGD is commonly char-

acterized by qualitative and quantitative changes in the glandular secretion.[6] Consequently, a decrease in lipid layer thickness is reasonably expected with increasing MGL. Because the lipid layer is an important component to stabilize the tear film,[50] correlation between MGL and non-invasive break-up time is expected. A loss of meibomian glands of more than 30% is reported to indicate MGD and dry eye.[8,20] To our knowledge, it is not exactly known how to interpret MGL observed by meibography. Some clinicians postulate that the apparent shortening of the meibomian gland may not be true atrophy in that the rest of the gland is faintly visible but may be deeper into the eyelid, further from the palpebral conjunctiva. However, Jester et al. were able to correlate histologically evaluated loss of normal acini in rabbits with complete loss of normal gland structure observed by meibography.[15] This loss of meibomian gland seems to be irreversible.[51]

FIGURE 10.

Evaluation of the meibomian glands using the Cobra fundus camera (CSO and bon Optic VertriebsgmbH) providing a close-up image (magnification ~10×) with optional digital magnification. A color version of this figure is available online at www.optvissci.com.

Clinical Practice

Meibography has been shown to be useful in the diagnoses of MGD. The simplest way to obtain meibography might be the use of white light and transillumination of the everted eyelid, observed by a slitlamp microscope. Unfortunately, this procedure might be uncomfortable for the patients and more technically challenging for the inexperienced examiner. However, non-contact meibography is a useful, quick, and patient-friendly method for obtaining information on the meibomian gland structure.[8,14]

As discussed, instruments offering non-contact meibography are now on the market in some regions of the world. One is the TOPCON® Slitlamp Microscope BG-4M which is equipped with an IR light and IR CCD camera; the others are the EyeTop® Topographer, Sirius® Scheimpflug Camera, and Cobra® Fundus Camera (CSO and bon Optic VertriebsgmbH)[30] and the Oculus Keratograph® (Oculus, Wetzlar, Germany). These multifunctional ophthalmic instruments use their built-in IR cameras for meibography.

While the TOPCON system allows different magnifications due the slitlamp microscope itself, the multifunctional ophthalmic instruments as well as the PNCM provides a close-up image of the entire only (Fig. 10) with the option of later digital magnification of the captured image. However, it seems to be important to analyze the relation of the meibomian glands loss to the entire eyelid.[8,41] Therefore, the option of higher magnification might be not essential in clinical practice. The advantage of the multifunctional instruments is that there is only need for a small software and optical update of already existing systems. The software associated with the mentioned topographer, Scheimpflug camera, and fundus camera (CSO and bon Optic) additionally includes computerized grading, which might make meibography more accessible in the daily routine (Fig. 11) and image documentation. Also, a well-trained technician can capture the images, freeing the doctor to focus on the clinical assessment and treatment.

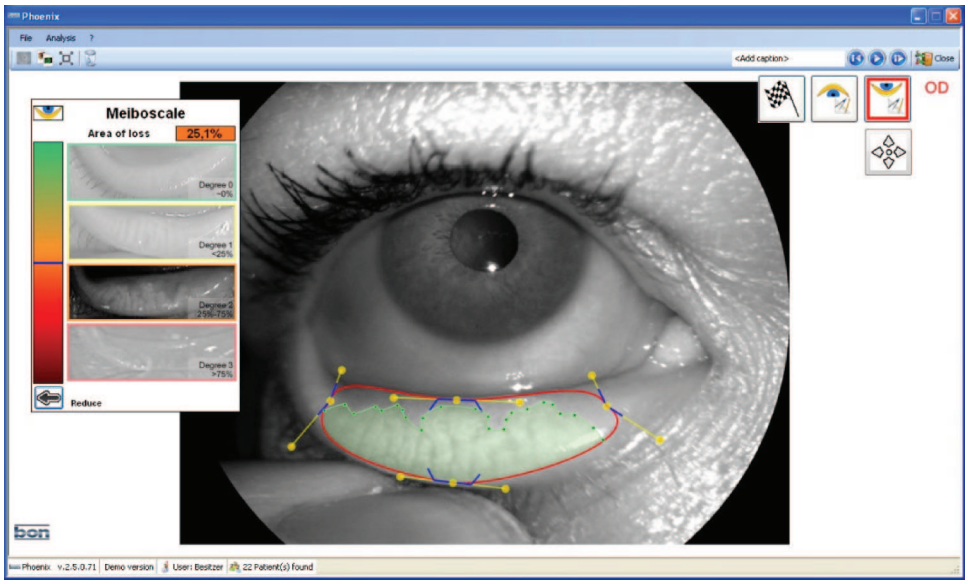

FIGURE 11.

Commercialized digital grading system in meibography (CSO and bon Optic VertriebsgmbH). A color version of this figure is available online at www.optvissci.com.

CONCLUSIONS

Meibography is a well-known option in the assessment of meibomian gland morphology, meibomian gland changes, and the diagnoses of MGD. Implementation of meibography in the daily routine should be considered because of its high specificity and sensitivity in the diagnoses of MGD and dry eye. In the past 40 years, there have been many techniques introduced; however, the introduction of non-contact meibography and commercialization of such instruments will advance meibography in research and clinical practice. Computerized classification of the meibomian glands has a promising future.

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